Bone Marrow Treatment of Mice Lethally Irradiated with Gamma-Rays under High Dose Rate. (III)

Effect of Isologous Bone Marrow Administered at Various Post-irradiation Days

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Isologous bone marrow cells were injected at various post-irradiation days to dd/s strain mice lethally irradiated with gamma-rays under high dose rate, and the survival rate, body weight change, and histology of hematopoietic organs were studied. Unless irradiated mice took an early down-hill course, a good survival rate was obtained even if bone marrow treatment was delayed up to four days after exposure. Treatment at 5 days still resulted in the survival of a small percentage of irradiated mice.

INTRODUCTION

In the previous reports[1,2] it was demonstrated that a good survival rate could be obtained when mice were treated with isologous or homologous bone marrow within several hours after high dose rate gamma-irradiation. How the survival rate will be when bone marrow is given at vavious times later than several hours after irradiation? It is practically important to know how long bone marrow treatment could be postponed without remarkably reducing the survival rate of lethally irradiated animals. The author gave isologous bone marrow to lethally gamma-irradiated mice within several hours and one, two, three, four and five days after exposure to study this problem.

MATERIALS AND METHODS

Dd/s strain female mice were used as bone marrow recipients and donors. The age of bone marrow donors was 5 to 6 weeks old. The age of mice used for irradiation in experiment No. 1 was 9 weeks and their mean body weight was 18.3 gm. Mice used for irradiation in experiment No. 2 were also 9 weeks of age, but their mean body weight was 20.8 gm., showing much better bodily growth than those in experiment No. 1. This difference in mean body weight may be due to that mice in experiment No. 1 grew in a rainy season. The method of obtaining bone marrow cells to be injected and the irradiation condition have been reported[1]. Mice to be irradiated and treated with bone marrow were divided into six groups; group-0, -1, -2, -3, -4, and -5. After mice were

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lethally irradiated with gamma-rays under high dose rate, isologous bone marrow was injected intravenously within several hours and at one, two, three, four and five days to group -0, -1, -2, -3, -4, and -5, respectively. The number of nucleated cells injected was approximately $5-12\times10^6$ and $8-12\times10^6$ in experiment No. 1 and No. 2, respectively.

RESULT

1) Survival Rate

Experiment No. 1: All irradiated control mice died within 11 days after exposure. As shown in Table 1 and Fig. 1, mice in the group-0 showed 50% survival rate, whereas the rate was rather considerably low in other treated

Table 1. Suvival rate of mice lethally gamma-irradiated and treated with isologaus bone marrow at various post-irradiation days.

Experiment No.	Sex	Dose-rate (r/min.)	Dose (r)	No. of nucl. cells injected	Group	No of surviving/No. of irradiated 7 days	14	20	30	60	*
1	female	3420	855	$5.0-11.7\times10^6$	0	5/8	5/8	4/8	4/8	4/8	50
					1	6/14	1/14	1/14	1/14	1/14	7
					2	4/14	0/14				0
					3	8/14	3/14	2/14	2/14	2/14	14
					4	6/14	2/14	1/14	1/14	0/14	7
					control	4/10	0/10				0
2	female	3360	896	$7.8-12.0\times10^6$	0	5/8	5/8	5/8	5/8	5/8	63
					1	8/8	7/8	7/8	6/8	6/8	75
					2	8/8	7/8	7/8	6/8	5/8	75
					3	7/8	5/8	4/7	2/7	1/7	29
					4	7/7	4/7	4/7	4/7	4/7	57
					5	6/8	1/8	1/8	1/8	1/8	13
					control	6/8	0/8				0

* % survival (30 days)

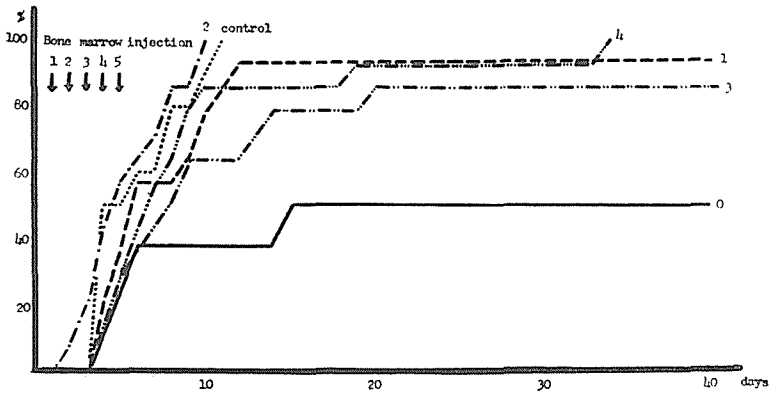

Fig. 1. Cumulative mortality of mice lethally gamma-irradiated and treated with isologous bone marrow at various post-irradiation days (Experiment No. 1).

groups and there was even a tendency for the death rate to increase within one or two days after bone marrow treatment. However, the fact that there were mice which survived for 30 days in the groups-1, -3, and -4 indicates that bone marrow treatment was effective, though slight in degree, in reducing mortality rate even if the treatment was postponed for four days.

Experiment No. 2: There were fairly good 30 day survival rates not only in group-0 but also in other treated groups except for groups-3 and -5, different from the result of experiment No. 1. Groups-1 and -2 showed even better survival rates than group-0. In group-3, which showed rather low 30 day survival rate, 20 day survival was fairly good, almost the same as that in group-0. This fact suggests that the recovering speed of hematopoietic organs in group-3 was not much different from that in group-0. The mortality curve in group-4 during a 30 day period was almost the same as that in group-0. Although the survival rate in group-5 during a 30 day period was much lower than those in other treated groups, it can be said that the bone marrow treatment at 5 days is still effective in reducing mortality rate of lethally irradiated mice (Fig. 2).

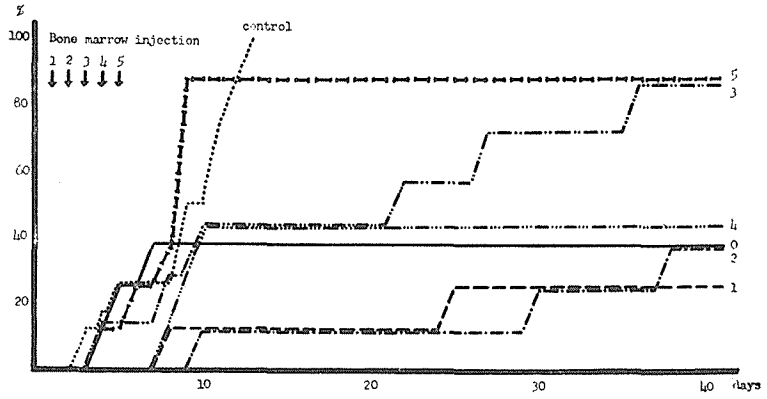

Fig. 2. Cumulative mortality of mice lethally gamma-irradiated and treatid with isologous bone marrow at various post-irradiation days (Experiment No. 2).

2) Body Weight Changes

Curves of mean body weight changes in experiment No. 1 were not written, because in no treated group other than group-0 more than three mice survived for the period of 30 days. Thus body weight changes in experiment No. 2 were described (Fig. 3). Both the treated mice and controls showed a rapid and continuous weight loss after irradiation. In the controls the weight loss continued until their death not showing a sign of recovery, while most mice in the treated groups showed stopping of their weight loss at 6 days followed by a gradual recovery. The body weight of mice in group-0 recovered to the pre-irradiation level (body weight at one day prior to irradiation) by the around 20th post-irradiation day. The body weight of mice in groups-1 and -2 recovered slower than that in group-0 despite better survival

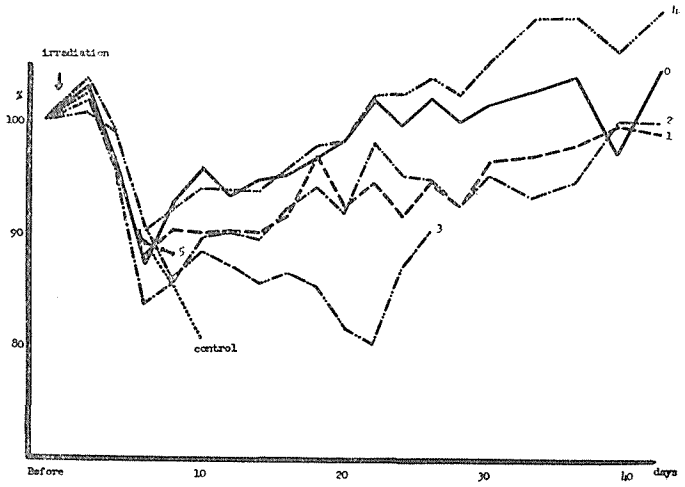

Fig. 3. Body weight changes of mice lethally gamma-irradiated and treated with isologous bone marrow at various post-irradiation days (Experiment No. 2).

rate in the former. This may be due to that more mice with decreasing weight died in group-0. The change of body weight of mice in group-4 was similar to that in group-0 as was in the case of mortality rate. The recovery of body weight of mice in group-3 was bad as was the survival rate. Since only one mouse survived during a 30 day observation period, mean body weight changes were written only for the first 8 days in group 5, and the surviving mouse showed 78 and 80% of the pre-irradiation weight (weight at one day prior to irradiation) at 30 and 42 days, respectively. As a summary, it might be stated that when bone marrow is given within 4 days after lethal irradiation one can expect to observe almost the same weight changes irrespective of the time of bone marrow injection.

3) Histological Findings

a) Bone marrow. Group-0: As described in Report I and II[1,2], the regeneration of the bone marrow began at 4 days in a few mice and at 5 days in many mice, and became nearly complete around at 8 days.

Group-1: After complete wasting a few small clumps of cells which consisted mainly of mature granulocytes and rarely of young bone marrow cells were seen at 4 and 5 days. It can hardly be thought that these cells are newly regenerated ones because of a large percentage of mature granulocytes. There are two other possibilities concerning their origin. One is that they have survived the irradiation damage and the other is that they are donor's injected cells entering and staying in the bone marrow cavity. The former possibility cannot be denied because a small number of mature granulocytes are still seen in the controls, lethally irradiated and non-treated mice. There is no evidence against the latter possibility either. A distict evidence of bone marrow regeneration was seen only at 6 days, the recovery seemed to proceed rather quickly and there was already an almost normal cellularity at 8

days in a few of them. In mice showing their bone marrow to be almost normal in cellularity at 8 days, the regeneration might have started at five days, starting at 6 days seems too late to give complete recovery in such a short period.

Group-2 : The earliest sign of regeneration was seen at 7 days and the regeneration became complete at 10 days.

Group-3 : Several clumps of cells consisting mainly of mature granulocytes were seen in the marrow cavity at 4 days after irradiation or one day after injection of isologous bone marrow, followed by the complete wasting for the subsequent two or three days. A distinct spotty regeneration was seen to begin at 7 days, and the recovery to normal cellularity was not complete before two weeks after exposure.

Group-4 : A small number of clumps of mature granulocytes and young marrow cells were noted, as in group-3, one day after injection of bone marrow. However, distinct regeneration did not manifest itself before 7 days. It took 3 to 4 weeks for the bone marrow to resume its pre-irradiation cellularity.

Group-5 and -6 : Distinct spotty regeneration of young marrow cells was observed at 9 days.

b) **Spleen.** The time of early regeneration of the red pulp was almost the same as or one day behind that of the bone marrow ; at 6, 8, 8, 7, and 9 days in group-1, 2, 3, 4, and 5, respectively. This slight delay in the early regeneration of the red pulp may be due to the difficulty of finding a small number of regenerating young cells in the spleen.

c) **Lymphnode.** No relationship between the recovery of the lymphnode and the time of bone marrow injection was noted.

d) **Thymus.** Although the speed of the recovery of the thymus varied from mouse to mouse, it can be stated that early regeneration of thymocytes began at 8 to 10 days irrespective of the time of isologous bone marrow treatment.

DISCUSSION

The difference in mortality rate between lethally gamma-irradiated and bone marrow treated mice and the controls, lethally irradiated but non-treated mice has been known due mainly to that in the speed of regeneration of hematopoietic organs[1,2]. Whereas no regeneration of hematopoietie organs took place before 9 days after lethal irradiation in the controls quick regeneration as early as at 4 days can be expected in mice treated with bone marrow within several hours after exposure. Is it an absolute necessity for hematopoietic organs to regenerate at as early as 4 or 5 days in order to have a good survival rate in lethally irradiated mice ? It was confirmed in the present study that a good survival rate could be obtained when regeneration of hematopoietic organs began by 6 or 7 days after exposure. The effect of delayed bone marrow treatment was very little or none in experiment No. 1. This may be due to that mice in experiment No. 1 were relatively retarded in their growth and were more susceptible to radiation injury, taking

early down-hill course. This explanation is supported by the short survival time after irradiation of the control mice in experiment No. 1 than those in experiment No. 2, being 6.3 and 9.1 days in the former and the latter, respectively. It is also assumed that injection of relatively large volume of bone marrow suspensions (0.5cc) is too much a load to mice taking already a down-hill course and it can be said that, in order for bone marrow treatment to be successful, mice should continue to be in a fairly good condition until there is a definite evidence of the early regeneration of hematopoietic organs.

The lag of time from the bone marrow injection to the beginning of bone marrow regeneration was three to five days 5, 4 and 3 days in groups-1 and -2, groups-3 and -5, and group-4, respectively. Delayed bone marrow treatment seems to give earlier regeneration of hematopoietic organs following the injection, though slight in degree. When bone marrow is given within 2 days after irradiation, there are still marrow cells remaining abundantly in the bone marrow cavity and when it is given later than 3 days the bone marrow of the host is completely acellular. The bone marrow cellularity and other factors such as the condition of circulation may influence the easiness of the injected marrow cells seed and grow in the bone marrow or spleen of the host.

There have been few articles dealing with the problem of the time of bone marrow treatment after lethal irradiation other than that of Lorenz *et al*[3]. in which they stated that the time of injection of bone marrow might be delayed in lethally irradiated mice for 3-4 days after exposure and that, with massive doses of bone marrow, this period could probably be extended a few more days and still one could get recovery of some animals. In the present study also a fairly good survival rate in lethally irradiated mice was observed to obtain even if the time of injection of bone marrow was delayed for as long as 4 days. Further, injection of a standard dose of bone marrow ($5\text{-}10\times10^6$ nucleated cells) at 5 days after exposure was still effective, though slight in reducing mortality rate. It is easily assumed that, even if bone marrow treatment was performed later than 5 days after exposure, a fairly good survival rate would be obtained if regeneration of hematopoietic organs begins before 7 or 8 days by injection of a massive dose of bone marrow. Urso *et al.* reported that the recovery of the bone marrow cellularity was quicker with the injection of massive doses of bone marrow[4]. Injection of bone marrow as late as 7 days after lethal irradiation would probably get survival of some mice if they do not take a down-hill course by that time.

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